ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.1103

Volume 130, pp. 1103–1110 *October–December 2015*

Three *Trichoderma* species associated with wood discoloration in South Korea

Seokyoon Jang, Yeongseon Jang, Gyu-Hyeok Kim & Jae-Jin Kim*

Division of Environmental Science & Ecological Engineering,
College of Life Sciences & Biotechnology, Korea University
5-1 Anam-dong, Seongbuk-gu, Seoul, 136-701, Korea

*Correspondence to: *jae-jinkim@korea.ac.kr*

Abstract — Three *Trichoderma* species previously recorded as new records from South Korea, *T. dorotheae, T. gamsii,* and *T. koningiopsis*, are described and illustrated.

Key words — ascomycetes, *Hypocrea*, phylogeny, taxonomy

Introduction

Trichoderma Pers. [= *Hypocrea* Fr.] is a cosmopolitan genus of fungi (*Hypocreaceae, Ascomycota*) comprising more than one hundred species (Druzhinina et al. 2006), which can be isolated from soil, wood, and various other substances, even including other fungi (Druzhinina et al. 2011).

In South Korea, about 20 *Trichoderma* species have been reported (Lee et al. 2003; Park et al. 2005, 2006; Cho et al. 2007; Huh et al. 2011). Ten *Trichoderma* species isolated from wood were identified by Huh et al. (2011), who examined the culture characteristics and analyzed the ITS and TEF sequences of the isolates; they reported three species—*Trichoderma dorotheae, T. gamsii*, and *T. koningiopsis*—as new records from South Korea but provided no microscopic descriptions. Here we confirm the identifications of these *Trichoderma* records through macro- and microscopical observations and an expanded phylogenetic analysis using translation elongation factor-1α (TEF) gene region sequences.

Materials & methods

Analysis of phenotype

Six isolates of the three *Trichoderma* species were obtained from the Korea University Culture Collection, Seoul, Republic of Korea (KUC). Cultures used for micromorphology were grown on corn meal dextrose agar (CMD: cornmeal 20 g, glucose 20 g, agar 18 g, distilled water 1000 ml), potato dextrose agar (PDA: Difco™ potato dextrose agar 39 g, distilled water 1000 ml) or Synthetic Nitrogenpoor or Nutrient poor agar (SNA: sucrose 0.2 g, glucose 0.2 g, KNO_3 1 g, KH_2PO_4 1 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, NaCl 0.5 g, agar 12 g, distilled water 1000 ml). The fungi were cultured at 25 or 30°C for 7d because their optimal growth temperatures were placed at 25–30°C (Huh et al. 2011). Microscopic characters were observed with an Olympus BX51 light microscope. Pictures of conidiophores and conidia were taken using the same microscope mounted with an Olympus DP20 microscopic camera. Measurements were made from 3% KOH mounts. At least 30 units of each parameter were measured for each collection. To make reliable data, 5% of the measurements from each end of the range were removed. The isolates were deposited at the National Institute of Biological Resources, Incheon, South Korea (NIBR).

Phylogenetic analysis

The TEF region was used for phylogenetic analysis of the *Trichoderma* species (Samuels et al. 2006). For three isolates (KUC1747, KUC5027, and KUC5063), TEF sequences were obtained from Huh et al. (2011). For the other three isolates (KUC3025, KUC5028, and KUC5064), DNA samples were extracted and the TEF region was amplified according to Huh et al. (2011). The TEF sequences were proofread and aligned with selected GenBank reference sequences using MAFFT 7.130 (Katoh & Standley 2013) and modified manually with MacClade 4.08 (Maddison & Maddison 2005). Datasets were tested by MrModeltest 2.3 using the AIC criteria with default options (Nylander 2004). The HKY + G model was chosen under the AIC criteria as a result of the test. Bayesian analysis was performed with MrBayes 3.2.1 (Ronquist et al. 2012). A phylogenetic tree was created according to Jang et al. (2012).

Taxonomy

Trichoderma dorotheae Samuels & Dodd, Stud. Mycol. 56: 112 (2006) Fig. 1a–c,j,k

Conidiophores lacking a discernible main axis and branches not obviously paired or unilaterally branched, re-branching to form second branches, which terminate in slightly divergent whorls of 3–4 phialides; conidiophores often arising from pustules, 2.0–2.5 µm wide. Phialides lageniform, swollen in the middle, 5.5–10.5 µm long, 2.5–3.5 µm at the widest point. Conidia broadly ellipsoidal, smooth, 3.2–4.0 × 2.2–3.5 µm.

Culture characteristics — Optimum temperature for growth on PDA and SNA 25°C. Colonies grown on SNA at 25°C remaining sterile. A white mycelium covering the 9 cm diam Petri plate at 25°C after 1 wk in darkness; No pigment diffusing through the agar; no distinctive odor. Colonies grown on PDA at 25°C in darkness forming conidia only after 96 h. A dense white mycelium covering the 9 cm diam Petri plate at 25°C after 1 wk in darkness; conidia moderate, bluish green. No pigment diffusing through the agar; no

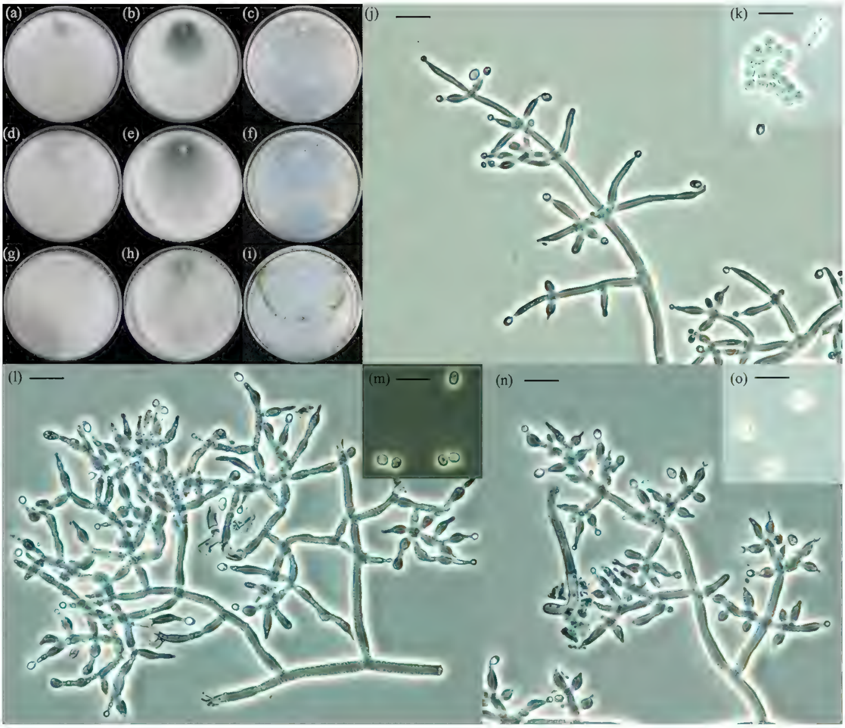

Fig. 1. ***Trichoderma dorotheae***: growth on (a) PDA; (b) CMD; (c) SNA. ***Trichoderma gamsii***: growth on (d) PDA; (e) CMD; (f) SNA. ***Trichoderma koningiopsis***: growth on (g) PDA; (h) CMD; (i) SNA. [All cultures in 9-cm-diam Petri dishes after 96 h at 25°C]. ***Trichoderma dorotheae***: (j) conidiophores; (k) conidia. ***Trichoderma gamsii***: (l) conidiophores; (m) conidia. ***Trichoderma koningiopsis***: (n) conidiophores; (o) conidia. Scale bar = 10 µm.

distinctive odor. Colonies grown on CMD at 25°C in darkness filling the Petri plate within 1 wk, colonies remaining sterile.

Distribution — New Zealand, Australia (Samuels et al. 2006), and South Korea.

Specimen examined: **KOREA, Jeollabuk-do,** Iksan-si, a log of *Pinus radiata*, August 2008, Jae-Jin Kim (NIBR-FG0000120470, culture KUC5027, GenBank HM534661).

Remarks — The TEF sequences of *T. dorotheae* KUC5027 (503 bp) and other *T. dorotheae* strains were monophyletic with high posterior probability support (PP = 1). *Trichoderma dorotheae* can cause light green discoloration, but wood-discoloration rate was low (Huh et al. 2011). The Korean isolate of *T. dorotheae* has a relatively high antagonistic ability against other wood-damaging fungi, so it has potential as a biological control agent (Lee et al. 2012).

Trichoderma gamsii Samuels & Druzhin., Stud. Mycol. 56: 168 (2006) Fig. 1d–f,l,m

Conidiophores more or less uniformly branched for a short distance, branches often arising from swollen nodes on the conidiophore, a single phialide forming at the tip, 2.0–3.0 µm wide. Phialides lageniform, at most slightly swollen in the middle, 7.0–10.5 µm long, 2.5–3.0 µm at the widest point, 1.5–2.5 µm at the base; Intercalary phialides common. Conidia ellipsoidal, smooth, 3.3–3.9 × 2.0–2.8 µm.

Culture characteristics Optimum temperature for growth on PDA and SNA 25–30°C; after 72 h on PDA colony radius reaching 50–52 mm, on SNA colony radius 39-42 mm. On PDA at 25°C and 30°C after 1 wk in darkness typically a dense white mycelium covering the 9 cm diam Petri plate; colonies remaining sterile. More or less strong coconut-like odor. On SNA at 25°C and 30°C covering the 9 cm diam Petri plate after 1 wk in darkness; conidia forming abundantly, grayish green, in broad sheets and sometimes confluent pustules sporadically produced, 1–2 mm diam. No pigment diffusing through the agar, no distinctive odor.

Distribution — Australia, Central Russia, Italy, U.S.A. (Jaklitsch et al. 2006), and South Korea.

Specimen examined: **KOREA, Jeollabuk-do**, Iksan-si, a log of *Pinus radiata*, August 2008, Jae-Jin Kim (NIBR-FG0000120471, culture KUC1747, GenBank HM534663).

Remarks — The most conspicuous characters of *T. gamsii* were its coconut-like odor and abundant aerial mycelia on CMD or PDA. As observation of conidiophores of *T. gamsii* on CMD or PDA was obscured by their aerial mycelia, most measurements were determined on SNA.

The macro- and micromorphological features of the Korean isolate agreed well with the protologue description, except for the protologue conidia being longer ((3.2–)4.0–5.0(–6.2) µm; Jaklitsch et al. 2006). The TEF sequences of *T. gamsii* KUC1747 (594 bp) and other *T. gamsii* strains were monophyletic with high support (PP = 1).

Trichoderma gamsii can cause green discoloration on wood surfaces with high wood-discoloration rate (Huh et al. 2011). On the other hand, *T. gamsii* has strong antagonistic properties against wood-rotting fungi and sap-staining fungi (Lee et al. 2012). *Trichoderma gamsii* KUC1747 produces 6-pentyl-α-pyrone that is a known antagonistic metabolite against sap-staining fungi (Hong et al. 2014).

Trichoderma koningiopsis Samuels, C. Suárez & H.C. Evans, Stud. Mycol. 56: 117 (2006) Fig. 1g–i,n,o

Conidiophores 2.5–3.5 µm wide, branches formed from the interior of pustules, the first branches rebranching or making phialides directly, the second branches tending to be paired; all fertile branches terminating in a whorl of phialides. Phialides lageniform, held in whorls of 2–5, sometimes several phialides arising from the same point and crowded, 5.5–7.5 µm long, 2.5–3.0 µm at the widest point. Conidia broadly ellipsoidal, smooth, 2.8–3.6 × 2.6–3.0 µm.

CULTURE CHARACTERISTICS Optimum temperature for growth on PDA and SNA 25–30°C. Colonies grown on PDA in darkness sometimes forming conidia after 48 h at 25 and 30°C in a concentric ring. A dense white mycelium covering the 9 cm diam Petri plate at 25°C and 30°C after 1 wk in darkness; conidia moderate, sometimes abundant at 30°C from some isolates. No pigment diffusing through the agar; rarely a faint coconut odor detected. Colonies grown on SNA in darkness for 96 h producing abundant aerial mycelium; conidia tending to be uniformly dispersed in the aerial mycelium in 1–2 broad concentric rings, sometimes forming cottony pustules. Conidial masses dark green. No pigment diffusing through the agar; no distinctive odor. Colonies grown on CMD in darkness a dense white mycelium covering the 9-cm-diam Petri plate within 1 wk at 25 °C. Conidial production restricted near the inoculated spot of the colony with a tendency to form highly compact to cottony, 1–2 mm diam pustules; Often long, entirely fertile branches visible in the pustules. Rarely a faint coconut odor detected.

DISTRIBUTION — Brazil, Canada, Cuba, Ethiopia, Ecuador, Germany, Ghana, Peru. Puerto Rico, U.S.A. (Samuels et al. 2006), Japan (Yabuki et al. 2014), and South Korea.

SPECIMENS EXAMINED: **KOREA, SEOUL,** Seongbuk-gu, Anam-dong, Korea University, chromated copper arsenate treated wood of *Pinus radiata*, 2003, Jae-Jin Kim (NIBR-FG0000120472, culture KUC3025, GenBank KJ652555); Mapo-gu, Sangam-dong, creosote contaminated soil, May 2007, Jae-Jin Kim (NIBR-FG0000120474, culture KUC5063, GenBank HM534662; NIBR-FG0000120475, culture KUC5064, GenBank KJ652554). **GYEONGGI-DO,** Gwangmyeong-si, creosote treated wood, 6 November 2007, Jae-Jin Kim (NIBR-FG0000120473, culture KUC5028, GenBank KJ652556)

REMARKS — The colony morphology and microscopic features of the Korean isolates agreed well with the protologue description, except for the protologue conidia being longer ((3.0–)3.5–4.5(–6.2) µm; Samuels et al. 2006).

The TEF sequences of *T. koningiopsis* KUC3025 (604 bp), KUC5028 (580 bp), KUC5063 (555 bp), and KUC5064 (553 bp) were placed inside the *T. koningiopsis* clade with high support (PP = 1). *Trichoderma koningiopsis* KUC5028, KUC5063 and KUC5064 were placed in the same subclade with high support (PP = 1). However, *T. koningiopsis* KUC3025 was clustered in another subclade with *T. koningiopsis* GJS 06-219 with high support (PP = 1). This intraspecific genetic variation was not associated with any phenotypic variation.

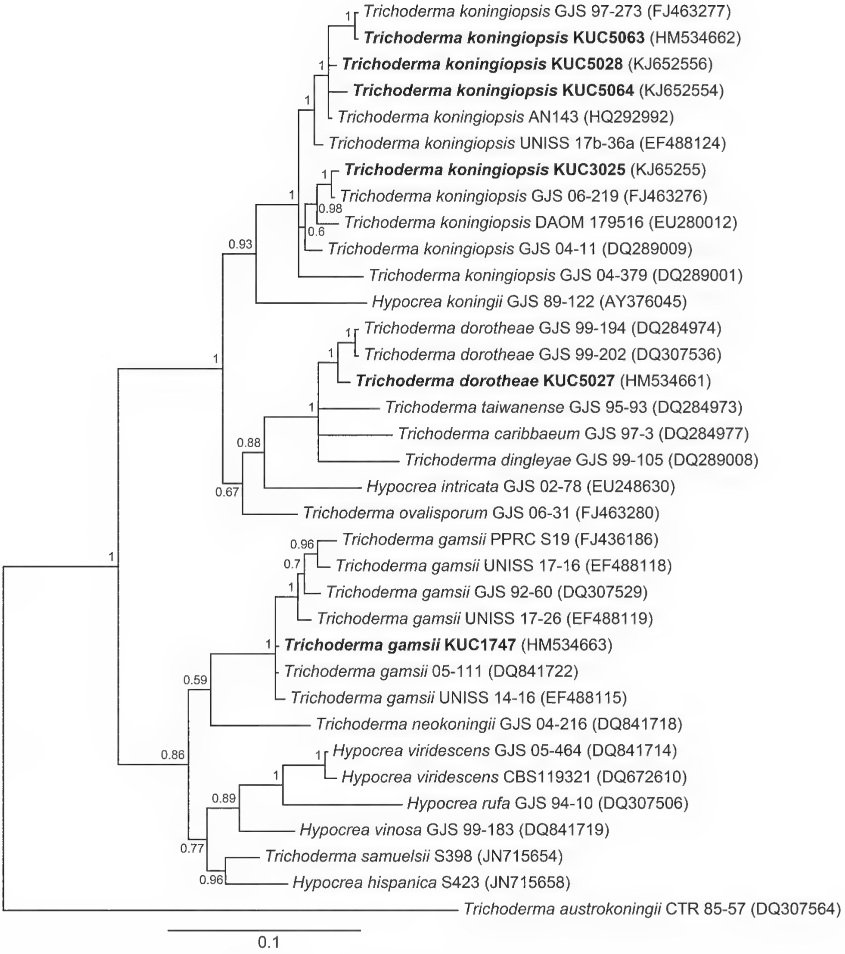
Trichoderma koningiopsis GJS 97-273 (FJ463277)
Trichoderma koningiopsis KUC5063 (HM534662)
Trichoderma koningiopsis KUC5028 (KJ652556)
Trichoderma koningiopsis KUC5064 (KJ652554)
Trichoderma koningiopsis AN143 (HQ292992)
Trichoderma koningiopsis UNISS 17b-36a (EF488124)
Trichoderma koningiopsis KUC3025 (KJ65255)
Trichoderma koningiopsis GJS 06-219 (FJ463276)
Trichoderma koningiopsis DAOM 179516 (EU280012)
Trichoderma koningiopsis GJS 04-11 (DQ289009)
Trichoderma koningiopsis GJS 04-379 (DQ289001)
Hypocrea koningii GJS 89-122 (AY376045)
Trichoderma dorotheae GJS 99-194 (DQ284974)
Trichoderma dorotheae GJS 99-202 (DQ307536)
Trichoderma dorotheae KUC5027 (HM534661)
Trichoderma taiwanense GJS 95-93 (DQ284973)
Trichoderma caribbaeum GJS 97-3 (DQ284977)
Trichoderma dingleyae GJS 99-105 (DQ289008)
Hypocrea intricata GJS 02-78 (EU248630)
Trichoderma ovalisporum GJS 06-31 (FJ463280)
Trichoderma gamsii PPRC S19 (FJ436186)
Trichoderma gamsii UNISS 17-16 (EF488118)
Trichoderma gamsii GJS 92-60 (DQ307529)
Trichoderma gamsii UNISS 17-26 (EF488119)
Trichoderma gamsii KUC1747 (HM534663)
Trichoderma gamsii 05-111 (DQ841722)
Trichoderma gamsii UNISS 14-16 (EF488115)
Trichoderma neokoningii GJS 04-216 (DQ841718)
Hypocrea viridescens GJS 05-464 (DQ841714)
Hypocrea viridescens CBS119321 (DQ672610)
Hypocrea rufa GJS 94-10 (DQ307506)
Hypocrea vinosa GJS 99-183 (DQ841719)
Trichoderma samuelsii S398 (JN715654)
Hypocrea hispanica S423 (JN715658)
Trichoderma austrokoningii CTR 85-57 (DQ307564)
1
1
1
1
0.98
0.6
0.93
1
1
1
1
0.88
0.67
0.96
0.7
1
1
0.59
1
1
0.86
0.89
0.77
0.96
1
0.1

Fig. 2. 50% majority-rule consensus tree of *Trichoderma dorotheae, T. gamsii, T. koningiopsis*, and allied species. Strains analyzed in this study are in bold font. GenBank accession numbers are in parentheses. Posterior probabilities ≥0.50 are shown above branches.

Even though *T. koningiopsis* caused green discoloration on wood surfaces, its wood-discoloration rate was low (Huh et al. 2011). *Trichoderma koningiopsis* KUC5063 showed strong antagonistic properties against wood-damaging fungi (Lee et al. 2012).

Phylogeny

The Bayesian tree of TEF region sequences was constructed to confirm the morphological identification. The aligned dataset of TEF contained 35 sequences and 600 characters. The three species are placed into Viride–Koningii section (Jaklitsch 2011).

Trichoderma gamsii was grouped with *T. neokoningii* Samuels & Soberanis (Fig. 2). Our phylogenetic result was similar to the study by Jaklitsch et al. (2006).

Trichoderma koningiopsis was monophyletic with *T. koningii* Oudem. [= *Hypocrea koningii* Lieckf. et al.], and *T. dorotheae* was placed in a monophyletic group with *T. caribbaeum* Samuels & Schroers, *T. dingleyae* Samuels & Dodd, and *T. taiwanense* Samuels & M.L. Wu. Again, the *T. koningiopsis* and *T. dorotheae* clades grouped together. However, phylogenetic trees in Jaklitsch (2011) showed that *T. koningiopsis* and *T. dorotheae* were monophyletic, but not with *T. koningii*. *Trichoderma koningii* was placed in the basal lineage below the two species (Jaklitsch 2011).

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2A10011390). This work was also supported by the project on survey and excavation of Korean indigenous species of NIBR under the Ministry of Environment, Republic of Korea, and by a Korea University Grant. We are grateful to Dr. Seonju Marincowitz and Dr. Toru Okuda for their valuable suggestions on the manuscript.

Literature cited

Cho H, Miyamoto T, Takahashi K, Hong S, Kim J. 2007. Damage to *Abies koreana* seeds by soil-borne fungi on Mount Halla, Korea, Canadian Journal of Forest Research 37: 371–382. http:/dx.doi.org/10.1139/x06-226

Druzhinina IS, Kopchinskiy AG, Kubicek CP. 2006. The first 100 *Trichoderma* species characterized by molecular data. Mycoscience 47: 55–64. http:/dx.doi.org/10.1007/s10267-006-0279-7

Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP. 2011. *Trichoderma*: the genomics of opportunistic success. Nature Reviews Microbiology 9: 749–759. http:/dx.doi.org/10.1038/nrmicro2637

Hong J-H, Lee J, Min M, Ryu S-M, Lee D, Kim G-H, Kim J-J. 2014. 6-Pentyl-α-pyrone as an anti-sapstain compound produced by *Trichoderma gamsii* KUC1747 inhibits the germination of ophiostomatoid fungi. Holzforschung 68: 769–774. http://dx.doi.org/10.1515/hf-2013-0171

Huh N, Jang Y, Lee J, Kim G-H, Kim J-J. 2011. Phylogenetic analysis of major molds inhabiting woods and their discoloration characteristics. Part 1. Genus *Trichoderma*. Holzforschung 65: 257–263. http:/dx.doi.org/10.1515/HF.2011.018

Jaklitsch WM. 2011. European species of *Hypocrea* part II: species with hyaline ascospores. Fungal Diversity 48: 1–250. http:/dx.doi.org/10.1007/s13225-011-0088-y

Jaklitsch WM, Samuels GJ, Dodd SL, Lu B-S, Druzhinina IS. 2006. *Hypocrea rufa/Trichoderma viride*: a reassessment, and description of five closely related species with and without warted conidia. Studies in Mycology 55: 135–177. http:/dx.doi.org/10.3114/sim.2006.56.04

Jang Y, Lee SW, Jang S, Lim YW, Lee JS, Kim J-J. 2012. Four unrecorded wood decay fungi from Seoul in Korea. Mycobiology 40: 195–201. http:/dx.doi.org/10.5941/MYCO.2012.40.3.195

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. http:/dx.doi.org/10.1093/molbev/mst010

Lee S, Hong S-B, Kim C-Y. 2003. Contribution to the checklist of soil-inhabiting fungi in Korea. Mycobiology 31: 9–18. http://dx.doi.org/10.4489/MYCO.2003.31.1.009

Lee J, Huh N, Hong JH, Kim BS, Kim G-H, Kim J-J. 2012. The antagonistic properties of *Trichoderma* spp. Inhabiting woods for potential biological control of wood-damaging fungi. Holzforschung 66: 883–887. http:/dx.doi.org/10.1515/HF.2011.187

Maddison D, Maddison W. 2005. MacClade 4: Analysis of phylogeny and character evolution. Version 4.08. Sunderland, MA: Sinauer Associates.

Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Park MS, Seo GS, Bae KS, Yu SH. 2005. Characterization of *Trichoderma* spp. associated with green mold of oyster mushroom by PCR-RFLP and sequence analysis of ITS Regions of rDNA. Plant Pathology Journal 21: 229–236. http:/dx.doi.org/10.5423/PPJ.2005.21.3.229

Park MS, Bae KS, Yu SH. 2006. Two new species of *Trichoderma* associated with green mold of oyster mushroom cultivation in Korea. Mycobiology 34: 111–113. http://dx.doi.org/10.4489/MYCO.2006.34.3.111

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. http://dx.doi.org/10.1093/sysbio/sys029

Samuels GJ, Dodd SL, Lu B-S, Petrini O, Schroers H-J, Druzhinina IS. 2006. The *Trichoderma koningii* aggregate species. Studies in Mycology 56: 67–133. http:/dx.doi.org/10.3114/sim.2006.56.03

Yabuki Y, Duncan I, Okuda T. 2014. Comparative study reveals unique features of the mycobiota in peat soils samples from Japan and Scotland. Mycoscience 55: 168–176. http://dx.doi.org/10.1016/j.myc.2013.08.002